

## Use of a quality assurance scheme in a long-term multicentric study of antibiotic susceptibility of *Neisseria gonorrhoeae*

### Abstract

Experience with a quality control programme for a multicentric collaborative study of antibiotic susceptibility of *Neisseria gonorrhoeae* is described. The Australian Gonococcal Surveillance Programme (AGSP) has monitored the antibiotic sensitivity of gonococci by surveying results of quantitative sensitivity tests performed in a number of centres throughout the country since 1981. A standardised system of testing by means of agar plate dilution was adopted to ensure uniformity of methods and results and to permit temporal and regional differences in antibiotic sensitivity to be identified and compared. A control programme was developed to provide quality assurance regarding each laboratory's performance. Over a period of nine years more than 3600 MIC determinations on control strains, examined as unknowns, were performed. An overall error rate of 3.1%, was observed. Higher error rates were noted in the beginning of the programme but these declined as the study progressed. In addition repeat and duplicate testing of strains was performed. The results of these tests also indicated an improvement in performance over the study period. Close attention to all aspects of the testing procedure reduced the number of errors recorded. At the beginning of the programme, various factors were investigated as possible causes of error. The formulation and preparation of media were found to be potential sources of result variation. Once these variables were controlled, lack of

experience with end-point determination and errors in preparation of dilutions of antibiotic solutions emerged as the commoner causes of discrepancy. In the experience of the AGSP, a long-term multicentric evaluation of gonococcal sensitivity can achieve accurate and reproducible results through standardisation of methods and application of a continuing quality assurance scheme.

In recent years a trend towards increasing resistance to both traditional and more recently introduced therapeutic agents has been observed amongst *Neisseria gonorrhoeae*. This has been manifested as an increase in the levels of chromosomally-mediated intrinsic resistance and also through the appearance of strains carrying plasmids coding for antibiotic resistance.

Antibiotic resistance in gonococci is often assessed by occasional surveys and reported in summaries of outbreaks or of sporadically occurring cases of treatment failures. The purpose of such investigations is to monitor levels of gonococcal susceptibility to antibiotics and to assist in the formulation of treatment regimens. However, long-term prospective studies of gonococcal susceptibility to antibiotics have the advantage of eliminating a number of variable factors which may affect these assessments. Some of these factors include temporary changes in patterns of gonococcal disease and transient alterations in antibiotic resistance. An example of a long-term prospective study is the former National Gonorrhoea Therapy Monitoring Study in the United States<sup>1</sup> where gonococcal isolates were referred to a central laboratory for susceptibility testing. Other national programmes rely on collation of data from multiple sources throughout a region or country.<sup>2</sup>

The value of any conclusion from investigations of this kind is dependent upon the accuracy of data from which it is drawn. If recommendations are to be based on the results of gonococcal sensitivity testing, it is of fundamental importance that the laboratory tests which provide this information are performed accurately. A number of difficulties arise with the sensitivity testing of gonococci because of the special growth requirements of the bacterium and the need for agar plate dilution techniques to determine all the

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various levels of intrinsic resistance that may be encountered. Additional problems occur with multicentric, as opposed to centrally-organised schemes, because a potential for discrepant results arises through methodological differences in the performance of quantitative sensitivity tests.

The Australian Gonococcal Surveillance Programme (AGSP) is a multicentric collaborative study of antibiotic resistance in gonococci which began in 1979 and has provided data on gonococcal sensitivity patterns and trends in gonococcal disease in Australia since 1981.<sup>3-5</sup> Over this period a uniform system for the performance of quantitative sensitivity tests was agreed upon and criteria for quality control and laboratory performance were proposed and developed. In this report, we elaborate on our experience of the influence of the type of cultural medium on the results of quantitative sensitivity tests and describe our observations on the organisation and value of quality control systems in surveillance schemes of this type.

### Materials and methods

The AGSP and methods initially employed for quality control have been described previously.<sup>3</sup> Briefly, the programme comprises major public health and hospital laboratories located in each state and territory in Australia. Gonococcal isolates obtained in or sent to these centres are identified, examined for their ability to produce beta-lactamase and tested for sensitivity to selected antibiotics by agar plate dilution techniques.

#### *Adoption of standardised technique*

The details of the standard method for determination of minimal inhibitory concentrations (MIC) of antibiotics used by the participants in the AGSP have been described.<sup>3</sup> The technique was based on WHO recommendations<sup>6</sup> except that a different test medium, Isosensitest agar (Oxoid) with added lysed horse blood, was used and an inoculum of  $10^4$  colony forming units (cfu) prepared from a fresh overnight subculture, employed. Prior to adoption of the standardised method, a wide variety of media were used in different laboratories throughout Australia. Differences in the basic composition of media, in the sources of the components in the growth supplements and in the preparation of media were noted. To evaluate the effect of media formulation on MIC testing, the MIC of penicillin for 18 control and wild type strains of gonococci was determined on six different media. The media used were the WHO medium,<sup>6</sup> the final medium chosen by the AGSP<sup>3</sup> and four other media in use in the laboratories of AGSP participants. The formulations of the various media were as follows: *medium 1*, Columbia agar base (Oxoid) plus 5% lysed horse blood (LHB); *medium 2*,

Isosensitest agar (Oxoid) plus 8% LHB; *medium 3*, Sensitest agar (Oxoid) plus 8% LHB; *medium 4*, GC agar base (Difco, USA) plus 1% haemoglobin and 1% Isovitalex (BBL, USA); *medium 5*, Columbia agar base plus 8% heated horse blood plus 1% Isovitalex; *medium 6*, Proteose peptone agar No. 3 (Difco) plus 1% haemoglobin and 1% Isovitalex. All aspects of the MIC determination in the evaluation were performed over the same period in the co-ordinating laboratory under standardised conditions by the same scientist in order to overcome any variation due to differences in antibiotic dilution and storage, preparation of inoculum, end-point determination or conditions of incubation.

In addition, the effect of the composition of the medium was further assessed in the co-ordinating laboratory by the determination of the MICs of penicillin for 184 strains of gonococci on WHO medium and the AGSP medium. Again, in this examination, a single operator performed all tests so that the only difference between the two series of MIC determinations was the composition of the media. In the standardised procedures, MICs of penicillin were determined over the range 0.004 to 2.0 mg/l. Gonococci were regarded as "fully sensitive" to penicillin if the MIC was equal to or less than 0.016 mg/l, as "less sensitive" over the range 0.06 to 0.25 mg/l and "relatively resistant" to penicillin for values of 1.0 mg/l and higher.

#### *Quality assurance procedures*

The co-ordinating laboratory despatched five selected strains of gonococci for MIC testing to each participant at monthly intervals nine or ten times a year. These strains comprised WHO reference strains, other stored strains whose MICs have been determined by repeated testing (see below), and recently isolated gonococci. Although beta-lactamase producing gonococci were classified separately and not subjected to formal determination of MICs of penicillin, strains of this type were included regularly in despatches so as to evaluate participants ability to detect them. Strains were stored as lyophilised cultures and at  $-70^{\circ}\text{C}$  in 30% glycerol in nutrient broth and were subcultured on to chocolate agar slopes and the resultant growth overlain with sterile liquid paraffin for despatch. These cultures were numbered sequentially and sent as unknowns. Individual strains were sent in different batches on more than one occasion and were also duplicated in the same batch to test the capacity of laboratories to reproduce results within and between batches of tests.

In the early years of the quality control programme the MIC values of the individual strains were accepted as those assigned to reference strains, or as those determined by the co-ordinating laboratory unless a significant discrepancy was observed. In practice no

such discrepancy occurred. As the scheme progressed it was possible to assign an "agreed value" for the MIC for each organism by determining the geometric mean of the MIC values obtained on the same strain and in different laboratories. By this means, a large panel of strains with predetermined MIC values over a range from 0.004 to 2.0 mg/l became available for testing.

Each laboratory was assigned a code number known only to that laboratory and the co-ordinating laboratory. Results of testing by individual laboratories were returned to the co-ordinating laboratory where the findings of all participants were recorded and tabulated. The results were then sent to each laboratory. Individual laboratories then identified their own results and compared their performance with those obtained elsewhere. In each calendar year, all results obtained were collated in the co-ordinating laboratory and reported to each participant for review and comment. Results of MIC testing were considered to be in agreement if the test value was within one two-fold dilution of the predetermined value. An erroneous result was one where a test value different from the predetermined value for the strain by two or more doubling dilutions was recorded and where this would thereby place the organism in a different sensitivity category.

## Results

**Effect of medium composition:** Table 1 summarises the variation in the MIC of penicillin recorded when 18 gonococcal isolates were tested on 6 different media. For purposes of comparison, the MIC values on various media are related to the results obtained on the AGSP medium (Medium 2). For 23 of the 90 determinations on other media, the MICs were identical to those on the AGSP agar. In another 45 instances, MICs on alternative media were one dilution higher, on a further 16 occasions two dilu-

Table 1 MIC of penicillin for 18 strains of *N. gonorrhoeae* obtained on AGSP medium (medium 2) compared with values obtained on five other media in use in Australia

No. of doubling dilutions difference in MIC value	Medium number*				
	1	3	4	5	6
Increase by 3				3	
Increase by 2	4		6	5	1
Increase by 1	9	9	9	9	9
Same	5	9	3	1	5
Decrease by 1					3

\*Medium 1, Columbia agar with 5% LHB; Medium 2, Isosensitest agar with 8% LHB; Medium 3, Sensitest agar with 8% LHB; Medium 4, GC agar with 1% LHB and 1% ISV; Medium 5, Columbia agar with 8% heated horse blood and 1% ISV; Medium 6, Proteose peptone agar No. 3 with 1% Hb and 1% ISV.

(LHB = lysed horse blood; Hb = haemoglobin; ISV = Isovitalex, Becton-Dickinson).

tions higher and in three instances three dilutions higher than the arbitrary standard. Three determinations gave readings one dilution lower than that on the AGSP medium. Media 1, 4 and 5 consistently gave higher readings for most strains whereas medium 3 gave values which approximated most closely to those obtained with the AGSP medium. Medium 6 gave a scatter of results above and below those for the standard medium.

Although the number of strains examined in each category was low, greater variation in results was seen in the seven strains classified as sensitive (MIC 0.004 to 0.016 mg/l) than in the nine isolates regarded as less sensitive (MIC 0.06 to 0.25 mg/l). The best correlation of results was seen for two strains whose MIC was 0.5 mg/l.

The figure shows a comparison between MICs of penicillin for 184 strains of gonococci obtained on WHO medium and AGSP medium. For 55 strains (29.8%), identical results were obtained on AGSP and WHO media. The MICs of 121 strains were higher on the WHO medium than on the AGSP medium, 93 (50.5%) by one doubling dilution, 26 (14.1%) by two doubling dilutions and two strains differed by a greater margin. Only eight strains (4.3%) had lower MIC values on WHO medium than they had on AGSP medium. The MICs of the strains were distributed in a bi-modal fashion.

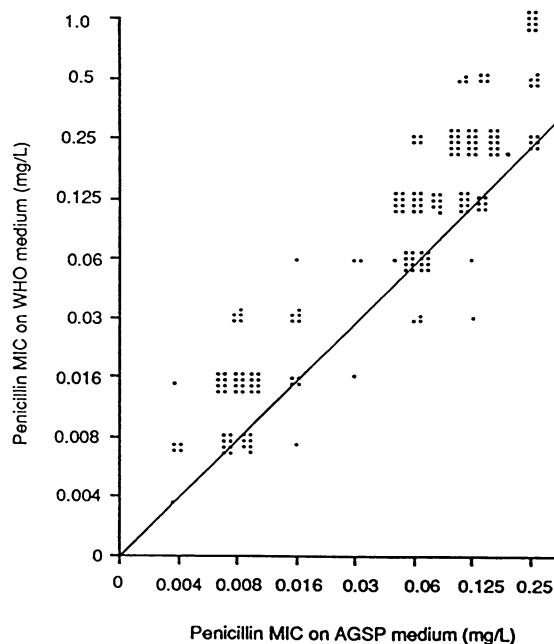


Fig Effect of medium composition on the determination of the penicillin sensitivity of 184 strains of *N. gonorrhoeae*. Comparison of results of MIC determinations on a medium used by the AGSP<sup>3</sup> and on a WHO-type medium.<sup>6</sup>

Table 2 Results of penicillin MIC determinations performed during quality control programme of AGSP 1980-88

Year	No. of strains despatched	No. of different strains in each year	No. of MICs performed	Errors*		Errors† on repeat testing		Errors‡ on testing strains in duplicate	
				No.	% all	No.	% repeat MICs	No.	% duplicate MICs
1980	45	32	344	16	4.6	6/144	4.1	1/44	2.2
1981	50	38	328	15	4.5	13/143	9.0	—	—
1982	45	18	341	25	7.3	15/282	5.3	2/34	5.8
1983	50	27	403	28	6.9	14/276	5.0	—	—
1984	50	29	441	10	2.3	4/300	0.01	0/18	—
1985	50	29	441	5	1.1	2/261	0.01	0/18	—
1986	50	29	439	2	0.01	2/293	0.01	0/18	—
1987	50	27	444	3	0.01	3/250	0.01	0/34	—
1988	48	26	434	10	2.3	8/329	2.4	0/45	—
Total 9 years	438		3615	114	3.1	67/2278	2.9	3/211	1.4

\*Error, MIC value differing by more than 1 doubling dilution from predetermined value.

†Error, MIC value differing by more than 1 doubling dilution from previous value obtained for that strain.

‡Error, MIC value of two strains tested at same time differing by more than one doubling dilution.

### Quality control surveys 1980-1988

In these surveys a total of 3615 MIC determinations were completed (table 2). The number of determinations varied slightly from year to year, increasing as the number of participants increased and altering when some strains were non-viable upon receipt. In the study a total of 114 results regarded as erroneous were reported representing 3.1% of MIC determinations. However, the proportion of errors was much higher in earlier years and a significant improvement was noted in the later years of the study.

Additionally, strains were sent on more than one occasion each year and, in most years, duplicate cultures of a strain were included in the same batches. Results of repeat and duplicate examination of strains are also shown in table 2. Repeated examination of the same strain resulted in 2278 MIC determinations being compared over the nine year period and a total of 67 (2.9%) erroneous results were noted. Of 211 duplicate examinations three results were incorrect.

Some participants performed particularly well over the entire period whereas, the results obtained by others differed from the agreed values for short periods. Analysis of events relating to the discrepant results suggested a number of different reasons for the variations. The single most apparent cause of discrepant results was error in determining end points. These were seen early in the study, as new laboratories joined the programme and as a consequence of staff rotation. With experienced staff none or few such problems occurred. Additionally, on a number of occasions there appeared to be loss of potency of stored antibiotic solutions. Repeat testing of another set of test strains with media containing freshly prepared antibiotics eliminated the discrepancy. In practice, it was found that the system of monthly despatch and return of tabulated results permitted early detection of errors and rapid correction of any problem.

### Discussion

In Australia in 1979, a diversity of systems for evaluating the sensitivity patterns of gonococci had developed owing to the influences of distance and a decentralised system of health care. In order to identify and compare regional differences in antibiotic sensitivity of gonococcal isolates, it was necessary to arrive at a common approach to laboratory procedures. Despite suggestions that a universal reference method for the performance of gonococcal sensitivity is desirable, as yet no agreed standard methodology has emerged. There was, however, a need for a uniform system of sensitivity testing if a multicentric programme was to be successful. Reyn *et al*<sup>7</sup> noted inter-laboratory variation in the level of MICs when 14 strains of gonococci were tested in three laboratories by their own methods. More recently, proficiency surveys in Britain<sup>8</sup> and the USA<sup>9</sup> have also noted the potential for discrepant results by laboratories performing gonococcal sensitivity tests. Reyn and her co-workers<sup>7</sup> observed that better agreement was obtained for results from different laboratories when a single technique was used, a finding confirmed in our earlier study.<sup>3</sup>

It is acknowledged that there are many sources of potential error in sensitivity testing but these can be reduced or eliminated if a uniform approach to all the steps involved in sensitivity testing is adopted. In our programme the recommendations of a WHO working group<sup>6</sup> published in 1978 were followed in most respects. However, an inoculum of 10<sup>4</sup> cfu, based on the recommendations of the report on the international collaborative study on sensitivity testing by Ericsson and Sherris,<sup>10</sup> was used. In other studies too, differing results were reported when inocula were varied.<sup>11</sup>

Our study, in keeping with the findings of others, noted particularly the influence of test medium on variation in MIC levels.<sup>7 10 11</sup> The medium used by

the AGSP tended to give lower readings than those obtained for the five other media with which it was compared including the WHO medium recommended in 1978.<sup>6</sup> A further comparison using the results of testing of 184 gonococcal strains confirmed the findings of Woodford and Ison,<sup>12</sup> namely, a tendency to higher readings if GC-type agars are employed. In addition the finding of Reyn *et al.*,<sup>7</sup> that these differences were not uniform over all MIC values, was confirmed. This meant that it is not practical to apply a "correction factor" to results to allow direct comparison of tests on different media. Woodford and Ison<sup>12</sup> commented on the uncertain reproducibility of batches of media based on GC agar formulations. We also noted an additional variable, namely the potential for variation in medium quality during preparation of this type of medium. In particular, there are several sources of GC agar base and difficulties were experienced with reconstitution of the haemoglobin powder. It should be remembered that this type of agar was formulated for the isolation of gonococci rather than as a sensitivity testing medium. Ultimately, the selection of the AGSP medium was influenced by availability, cost and ease of production as well as its demonstrated performance.

The adoption of a standard technique does not guarantee uniformity of results. We therefore sought as well to identify potential sources of error within and between laboratories and to develop quality control programmes applicable to our long-term study. Reyn *et al.*<sup>7</sup> introduced the concept of reference strains and in the AGSP programme, a large number of such strains were defined and used for the detection of any significant variation from expected results. It is our experience that most errors arose from problems associated with end point determination. The inclusion of strains with known MIC values in each batch of tests serves as a check on a number of factors such as the growth-promoting capacity of the medium. However, there is always a tendency, no matter how objective the observer is, to match the observed to the expected end point when "known" controls are used. We have found that a more effective method of detecting errors in performance is to include external controls with pre-determined values which are, however, unknown at the time of testing.

The AGSP study also demonstrates that such quality assurance schemes not only have the capacity to identify errors in performance but also result in higher standards of laboratory performance over time through correction of observed deficiencies. The number and percentage of errors recorded in this study were low, despite the use of stringent performance standards.

One consideration sometimes overlooked is that the distribution of MICs of penicillin for gonococci is

predominantly bimodal rather than continuous.<sup>3,13</sup> Most strains are not beta-lactamase producing gonococci and fall into either the fully sensitive or the less sensitive group. A more recently defined category comprises strains relatively resistant to the action of penicillin by virtue of chromosomally-mediated intrinsic resistance to the antibiotic. Because of recognised differences in different countries in the numerical values assigned to these various categories, it may be more useful for groups performing gonococcal susceptibility testing to assign strains to a particular category rather than report an MIC value. In the absence of an agreed standard procedure reference strains can be used to define these groupings. This procedure has been followed in our programme in the regular reports of our findings in local bulletins.<sup>14</sup>

With our programme we have the additional facility to directly and confidently compare actual MIC values of strains obtained in different centres. By these means, we have been able to identify regional differences in levels of intrinsic resistance in Australia,<sup>4</sup> a process that is only possible when equivalent data are available from the different centres.

The benefits of longitudinal studies on the sensitivity of gonococcal isolates have been emphasised previously. These include the provision of an indicator system for emergence of antibiotic resistance amongst gonococci and a scientific basis for establishing treatment regimens. Further, we have been able to use data from our study to monitor changing trends in the epidemiology of gonorrhoea and, by implication, other sexually transmitted diseases.<sup>5</sup> The validity of the data ultimately depends upon the accuracy of results. The Australian experience is that errors due to technical variations can be largely eliminated and that longitudinal multicentric studies can provide reliable and useful information.

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